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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification** 6 :
C12Q 1/00, G01N 27/327, C12Q 1/54

A1

(11) **International Publication Number:** **WO 95/21934**

(43) **International Publication Date:** 17 August 1995 (17.08.95)

(21) **International Application Number:** PCT/GB95/00265

(22) **International Filing Date:** 10 February 1995 (10.02.95)

(30) **Priority Data:**
9402591.3 10 February 1994 (10.02.94) GB

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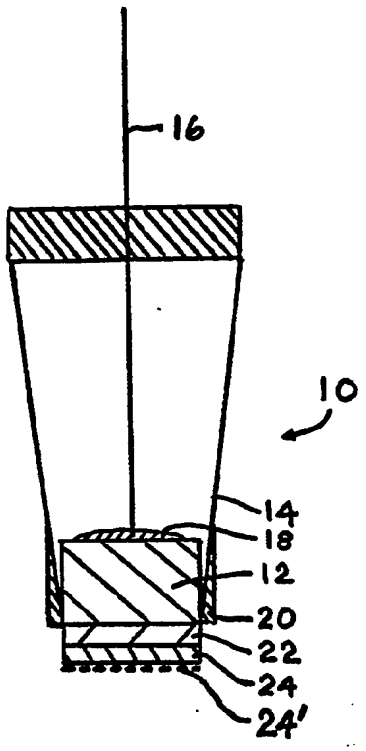
(81) **Designated States:** CA, FI, GB, JP, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) **Title:** HEXACYANOFERRATE MODIFIED ELECTRODES

(57) **Abstract**

Amperometric biosensors, e.g. for determining blood glucose, employ electrodes, preferably of graphite or glassy carbon, which have been modified by electrochemical deposition of a Prussian blue - type coating. Such electrodes are capable of amperometric detection of hydrogen peroxide, largely unaffected by potential interferents including ascorbate and paracetamol. A hydrogen peroxide-generating enzyme may be immobilised on the electrode or otherwise coupled to it so that the system becomes a biosensor.



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Hexacyanoferrate Modified Electrodes

Technical Field

This invention relates to hexacyanoferrate (or Prussian blue) modified electrodes which may be coupled with enzymes, particularly oxidoreductase enzymes e.g. glucose oxidase for the amperometric determination of analytes such as glucose.

Background Art

Amperometric enzyme electrodes have proved to be an important approach for sensing in medicine, in the food industry and for environmental monitoring. (See, for example, Newman, J.D. and Turner, A.P.F., Essays in Biochemistry, 27, 147-159, 1992.)

Amperometric biosensors generally work by monitoring oxygen consumed or hydrogen peroxide produced during enzymatic oxidation of the analyte. An artificial electron acceptor (mediator) may be used instead of the natural electron acceptor oxygen (see e.g. Cass *et al.*, Anal.Chem, 56, 667-671 1984; Matthews *et al.*, Diabetic Medicine, 5, 248-252, 1987). This enables the sensor to be operated at a low potential thus reducing the effect of interfering compounds commonly observed at the high operating potential employed for hydrogen peroxide detection.

The majority of experimental prototype implantable biosensors based on hydrogen peroxide detection utilise platinum as the base electrode as opposed to the cheaper alternative carbon, the reason being that

hydrogen peroxide can be detected at a lower potential at platinum than at carbon.

There has been some academic work on the electrochemistry of electrodes modified with Prussian blue, by Itaya and co-workers. Thus Itaya *et al*., Electrochem. Sci. and Technol., 129(7), 1498-1500, (1982) disclose the preparation of Prussian blue ("PB") modified electrodes by electrochemical deposition from ferric-ferricyanide solutions onto platinum, glassy carbon or SnO_2. Itaya *et al*., J.A.C.S., 106, 3423-3429 (1984) describe investigations on PB-modified electrodes including catalysed hydrogen peroxide oxidation at rotating PB-modified glassy carbon disc electrodes.

Disclosure of Invention

The present invention results from the discovery that modified electrodes of the same general type as those prepared by Itaya *et al*. have particular advantages for certain analytical applications, particularly in biosensors.

In one aspect the invention provides an enzyme electrode comprising:

(i) a modified electrode comprising:

(a) a conductive element having a surface; and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface; and

(ii) an enzyme retained on or adjacent said

coating, said enzyme being selected such that a substrate or product thereof is capable of being electrochemically oxidised or reduced at said modified electrode.

In a second aspect the invention provides an amperometric biosensor comprising a cell for receiving an analyte, and electrodes for contacting analyte in the cell, said electrodes comprising a sensing electrode, a standard electrode and, optionally, a counter electrode, and wherein said sensing electrode comprises a modified electrode comprising:

(a) a conductive element having a surface; and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface; said biosensor including an enzyme selected such that a substrate or product thereof is capable of being electrochemically oxidised or reduced at said modified electrode; said enzyme being disposed in relation to the modified electrode so that, in the operation of the biosensor, the enzyme affects the amount of said substrate or product and thereby affects a signal current of said cell.

The enzyme may be retained on or adjacent said coating of the modified electrode which thus constitutes an enzyme electrode.

The biosensor may include means defining a fluid flow path through said cell, and wherein said enzyme is

disposed in the flow path upstream of the cell so as to affect the composition of the fluid flow reaching the cell.

A preferred type of embodiment is adapted for determining glucose in whole blood, serum or plasma.

In a third aspect the invention provides a method of determining the amount of an analyte in a sample comprising contacting a solution comprising said analyte with an enzyme electrode according to the first aspect and with a standard electrode, applying a potential between said electrodes, and monitoring the electrical current. The analyte may be a substrate for the enzyme, or an inhibitor or an activator thereof.

In a fourth aspect the invention provides a method of determining hydrogen peroxide in an analyte solution in the presence of one or more potentially interfering substances selected from ascorbate, uric acid and 4-acetamidophenol ("paracetamol" or "acetaminophen"), comprising contacting said analyte solution with a modified electrode comprising:

(a) a conductive element having a surface and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface; and with a standard electrode, applying a potential between said electrodes, and monitoring the electrical current.

In a fifth aspect the invention provides a method of determining an analyte by means of an affinity

reaction using an enzyme label, wherein the amount of enzyme label is detected amperometrically by determining a substrate or product thereof by means of a modified electrode comprising:

(a) a conductive element having a surface and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface.

An electrode as used in preferred embodiments of the invention can be much cheaper than a platinum electrode (e.g. using carbon as the underlying conductor) and can offer excellent resistance to interfering substances.

A modified electrode for use in the present invention may be a conductor with a PB coating applied as disclosed by Itaya in the papers referred to above. Alternatively the conductor, preferably of carbon, may be placed in a solution of hexacyanoferrate ("HCF") II and/or III and connected into an electrolytic circuit with another electrode and a source of potential. Preferably the potential of the conductor is cycled between a low or zero value and a positive value. Electrochemical deposition can provide suitable reaction kinetics to allow the formation of the complex. Prussian blue analogues display characteristic cyclic voltammagrams, hence cyclic voltammetry can be used to determine if film deposition has been successful. A simple test to see if the

deposited layer is suitable for biosensor application would be to poise the electrode at a relevant operating potential (amperometry) and immerse it in a solution containing the biological element. Once the background current has stabilised, aliquots of the substrate for the biological element can be introduced into the cell and the change in current as a result of this addition can be recorded. If current change is proportional to the substrate concentration then the modified electrode is suitable.

Some embodiments of the invention will now be described in greater detail with reference to the accompanying drawings.

Brief Description of Drawings

Fig. 1a and b are schematic sectional views of two embodiments of enzyme electrodes of the invention.

Fig. 2 is a schematic sectional view on a smaller scale of an analytical cell including the electrode of Fig. 1a.

Fig. 3 is a cyclic voltammogram of a HCF-modified graphite electrode.

Fig. 4 is a calibration graph of glucose concentration versus current for the cell of Fig. 2.

Figs. 5-7 are bar charts showing the response of bare and HCF-modified electrodes to various circumstances.

Figs. 8 and 9 are graphs showing the effects on glucose determination, using modified and unmodified

electrodes, of ascorbate and 4-acetamidophenol in concentrations typically found in the blood of persons taking therapeutic doses.

Fig. 10 is a bar chart showing the effects of high concentrations of ascorbic acid and 4-acetamidophenol on glucose determination using modified and unmodified electrodes.

Fig. 11 is a cyclic voltammogram for demonstrating electrode stability.

Modes for Carrying Out the Invention

Materials and Methods

Glucox PS (E.C.1.1.3.4., 35000 International Units/g from *Aspergillus Niger*) was obtained from Rhone Poulenc Chemicals (Stockport, Cheshire, UK). Glucose, dipotassium hydrogen phosphate, potassium dihydrogen orthophosphate, potassium chloride, potassium hexacyanoferrate III and l-ascorbic acid were all of analytical grade and purchased from BDH (Poole, Dorset, UK). 1-Cyclohexyl-2-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate for enzyme immobilisation was purchased from Sigma (Dorset, Poole, UK). Uric Acid 99%, 4-acetamidophenol 98% from Aldrich Chemical Co., (Gillingham, Dorset). Platinum s.w.g.26, 0.44mm wire was purchased from BDH (Poole, UK). Spectrotech purity graphite rod 4.572mm diameter X 304.8mm long was purchased from Johnson Matthey, Materials Technology (Royston, UK).

The autolab and General Purpose Electrochemical

System software package supplied by Windsor Scientific Limited, (Berkshire, UK) was employed for all voltammetric experiments. A Ministat potentiostat and CR 600 JJ Instruments chart recorder were used in all amperometric measurements. In both cases a three electrode cell was used. All potentials quoted are with respect to a saturated calomel electrode (S.C.E). Platinum wire was used as the counter electrode.

Electrodes 10 as shown in Fig. 1a were prepared as follows.

Graphite rods 12 (1cm in length) were washed in a large stirred volume of boiling deionised water for 15 minutes. The rods were then rinsed in acetone and left to air dry in a drying cabinet at 80 degrees Celsius for approximately two hours. The rods were sealed in plastic capillary pipettes 14 and wire 16 was threaded through one end and bonded with silver loaded epoxy resin 18 to form a contact. Araldite(RTM) epoxy resin 20 was used to insulate the sides of the electrodes. All electrodes were polished prior to use.

The working electrode was cycled fifteen times between 0-2.5V vs SCE at a scan rate of 0.2V/s in potassium hexacyanoferrate (III) dissolved in deionised water (0.1M, 5ml) to deposit a layer 22. The ferricyanide solution was continually stirred during cycling. The modified electrodes were washed in a large, stirred, volume of water until the initial pale blue colour was no longer observed. (These electrodes

were used for experiments not requiring enzyme activity.)

A cyclic voltammogram of the modified electrode in 0.1M potassium chloride was obtained, at a scan rate of 10mV/S (Figure 3).

Glucose oxidase was immobilised onto the surface of the modified carbon electrodes using carbodimide immobilisation. A modified electrode was placed in a beaker containing stirred 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide (0.15M, 5ml) in sodium acetate buffer (0.1M, pH5.5) for ninety minutes. The electrodes were washed thoroughly with deionised water. Glucose oxidase (10μl 200mg/ml) in sodium phosphate buffer (0.02M, pH7) containing potassium chloride (0.1M) was deposited onto the electrode surface and left to dry for four hours, to produce an immobilised enzyme layer 24. A semipermeable membrane 24' coating the enzyme layer 24 was produced as follows. Using micropipettes, two layers of Nafion solution and two layers of polyurethane solution were applied. The Nafion solution was 5% in aliphatic alcohols plus water, as supplied by Aldrich. The electrode was dried for 5 minutes after the first layer, and for 1 hour after the second layer. The polyurethane solution was 10% in 9:1 w/v THF/DMF. The coated electrode was stored at 4°C overnight to dry. The dried electrodes were stored in potassium phosphate buffer at 4 degrees Celsius until use.

Type 2 'minature' electrodes as shown in Fig 1b were prepared by a similar process, starting from "pencil lead" carbon rods. Each rod 112 was first modified with HCF (III), producing a film 122 over an end region. To produce enzyme electrodes, layers of enzyme 124 and (preferably) semipermeable membrane were also applied, over the HCF layer. Supports for the rods were produced by cutting about 5cm of the narrow end off a "C10 Pipetteman" tip. A rod 112 was fed (modified end first) into the truncated tip 114, from the wide end. Wire 116 was fed part way, just into the well defined in the tip. A small plug of epoxy resin 120 was placed near the bottom of the well, for sealing. A larger plug 118 of silver-loaded epoxy resin closed off the top of the device and connected the wire 116 to the rod 112.

Amperometric Determination of Glucose

The enzyme electrode response to glucose was determined by immersing the electrode in 10ml of stirred potassium phosphate buffer 25 (0.02M, pH7) containing potassium chloride (0.1M) in a water-jacketed (26) cell, thermostated at 25 degrees Celsius. The cell contained a saturated calomel electrode (SCE) 28 and a platinum wire counter-electrode 30. A potential of +450mV vs SCE was applied to the electrode. Once the background current had stabilised, successive 2µl injections of glucose (1M) in potassium phosphate buffer (0.02M, pH7) containing potassium

chloride (0.1M) were introduced into the cell giving a concentration range of up to 2.8mM. The steady state response after each addition was recorded. A calibration plot for the glucose sensor is shown in figure 4.

Amperometry was also used to investigate the effect of hydrogen peroxide (Figures 5 and 6), ascorbate, uric acid and 4-acetamidophenol (Figure 7) at a HCF (III) modified electrode (with no enzyme) and at an unmodified electrode.

A typical cyclic voltammogram of these modified electrodes (Figure 2) showed two redox couples, the first at about 0.224V and 0.148V and the second at 0.923V and 0.798V. Similar electrochemistry was observed with electrodes which were modified using solutions of HCF(II) and HCF(II)/HCF(III). The redox potentials of the modified electrodes concurred with those cited in the literature for Prussian blue (Itaya *et al.*, 1982) and therefore leads us to conclude that the modified electrodes may bear layers of a Prussian blue analogue.

In one application, glucose oxidase was immobilised on to the surface of these modified electrodes and the resulting enzyme electrode was used to monitor glucose. A typical sensor of this type exhibited a linear range up to 1.5mM glucose and sensitivity of 4.97μA mmolar $^{-1}cm^{-1}$. The use of outer membranes such as the polyurethane coating used herein,

was found to extend the linearity. The membranes act to restrain diffusion.

Two experiments were performed to determine whether the amperometric glucose-generated signal was a mediated response or oxidation of hydrogen peroxide. Firstly, the direct oxidation of hydrogen peroxide at 450mV vs SCE at an unmodified electrode was compared to that at an HCF(III) modified electrode. The response of the unmodified graphite electrodes ("-HCF") to hydrogen peroxide (3.32mM) was negligible relative to the response at a modified electrode ("+HCF")(Figure 5). Additionally, the glucose sensors displayed a reduced response to glucose (3.32mM) in a nitrogen saturated environment ("N_2") relative to an oxygen saturated environment ("O_2") (Figure 6). (To achieve saturation, nitrogen or oxygen was bubbled through the liquid in the cell to displace other gases, eg for 1 hour.) Both these findings lead us to propose that the glucose response of the sensor is via electrocatalytic oxidation of hydrogen peroxide by the modified electrode.

The response of common interferents (100μM) such as ascorbic acid and 4-acetamidophenol at a modified electrode ("+HCF") was similar to that observed at an unmodified electrode ("-HCF") (Figure 7). The difference in response of uric acid (100μM) at unmodified and modified electrodes was slightly more difficult to distinguish because of the large errors

incurred in this particular determination (Figure 7). However, from Figure 7 we deduce that any electrocatalytic effect the modified carbon electrode has on uric acid is negligible.

Ascorbic acid/ ascorbate and 4-acetamidophenol are very important potential interferents in areas of great commercial importance. Their effect at both physiological and pharmacological levels (that is, levels as might occur in blood fluids of patients taking normal doses or excessive amounts, respectively) was also investigated at the miniature unmodified and modified carbon base electrodes (ie electrodes as shown in Fig 16). The electrodes were operated at the poised potential in 5mM buffered glucose (15ml) until the current reached steady state ("100%"). Three successive additions of either 0.1M ascorbic acid or 0.1M 4-acetamidophenol (7.8µl) were introduced into the cell giving a 50µM increment at each addition. For concentrations representative of pharmacological levels 120µl of the interferent stock solution was added to the cell giving a final concentration of 800µM. The increase in steady state current was noted and the percentage change in current as a result of addition to the interfering substance was calculated as follows:

$$\% \text{ change in current} = \frac{\text{Increase in Current}}{\text{Current in 5mM Glucose}} \times 100$$

Figures 8-10 show the percentage changes in current caused by interferents.

Figure 8 shows that physiological levels of ascorbic acid produced no significant interference in sensor output when added to 5mM glucose at miniature modified carbon base electrodes. However, an approximately 10-fold increase in current was observed at miniature unmodified carbon base electrodes operated under similar conditions. Fig. 9 shows that a 1-2-fold increase in sensor output was observed when normal levels of 4-acetamidophenol were added to 5 millimolar glucose at miniature modified carbon-base electrodes The same effect was observed at pharmacological levels (800μM) of ascorbic acid and paracetamol (Fig. 10).

Itaya *et al.*, 1984, observed that oxygen reduction at a Prussian blue derivatised electrode produces water (four electron reduction), as opposed to hydrogen peroxide (two electron reduction) which is the product typically observed at a base metal electrode. On the basis of this observation they have hypothesised that the local three dimensional distribution of iron centres within a Prussian blue derivative layer might act as a multi-electron source which is the reverse of the one electron nature of ferricyanide reactions in solution.

Furthermore these workers have suggested that this electrocatalytic effect might be observed only with substrates small enough to penetrate the Prussian blue

lattice where electron transfer reactions can occur.

Our results appear to conform to this hypothesis. Hydrogen peroxide molecules may by virtue of their small size be able to penetrate the modified lattice and undergo subsequent oxidation whereas larger molecules such as ascorbic acid may be hindered from penetrating the lattice. Additionally in the case of anions such as ascorbic acid, charge repulsion may also contribute to the failure of such ions to reach the iron centres.

The modified electrodes were surprisingly stable and offer the commercially important advantages of operation at low potential and selectivity. The apparent absence of leaching will have important implications for *in vivo* monitoring of analytes such as glucose, where stable, continuous output of the sensor is essential. This invention may also find general applications in biosensors and chemical sensors.

Evidence of the electrode stability is provided by Fig. 11. HCF(III) modified graphite disc electrodes were repeatedly cycled at 200 mV/s in 0.1M potassium chloride. Traces are shown for scan nos 2, 15 and 30. It can be seen that there is remarkably little change.

Different embodiments may use different enzymes. Thus a wide range of analytes could be measured e.g. glucose, lactase, alcohol and many more.

Carbon is inexpensive and particularly amenable to mass production technology e.g. screen printing, thus

improving the electrochemistry of H_2O_2 at carbon so that it is as good as at platinum is useful.

Mediators may also exhibit good electrochemistry at this surface, so that the utility of such electrodes is not restricted to systems generating H_2O_2.

CLAIMS

1. An enzyme electrode comprising:

(i) a modified electrode comprising:

(a) a conductive element having a surface; and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface; and

(ii) an enzyme retained on or adjacent said coating, said enzyme being selected such that a substrate or product thereof is capable of being electrochemically oxidised or reduced at said modified electrode.

2. An enzyme electrode according to claim 1 wherein said enzyme is an oxidoreductase enzyme which produces or consumes hydrogen peroxide, and said modified electrode is capable of oxidising hydrogen peroxide.

3. An enzyme electrode according to claim 2 wherein said enzyme is a glucose oxidase.

4. An enzyme electrode according to any preceding claim wherein said enzyme is immobilised on said coating of said modified electrode.

5. An enzyme electrode according to any preceding claim wherein said conductive element comprises carbon.

6. An enzyme electrode according to claim 5 wherein said conductive element comprises graphite or glassy carbon.

7. An enzyme electrode according to any preceding claim wherein the conductive element is provided by a conductive coating on a substrate.

8. An amperometric biosensor comprising a cell for receiving an analyte, and electrodes for contacting analyte in the cell, said electrodes comprising a sensing electrode, a standard electrode and, optionally, a counter electrode, and wherein said sensing electrode comprises a modified electrode comprising:

(a) a conductive element having a surface; and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface; said biosensor including an enzyme selected such that a substrate or product thereof is capable of being electrochemically oxidised or reduced at said modified electrode; said enzyme being disposed in relation to the modified electrode so that, in the operation of the biosensor, the enzyme affects the amount of said substrate or product and thereby affects a signal current of said cell.

9. An amperometric biosensor according to claim 8 wherein said enzyme is retained on or adjacent said coating of the modified electrode which thus constitutes an enzyme electrode.

10. An amperometric biosensor according to claim 8 wherein said enzyme electrode is according to any of

claims 1-7.

11. An amperometric biosensor according to claim 8 which includes means defining a fluid flow path through said cell, and wherein said enzyme is disposed in the flow path upstream of the cell so as to affect the composition of the fluid from reaching the cell.

12. An amperometric biosensor according to any of claims 8-11 adapted for determining glucose in whole blood, serum or plasma.

13. A method of determining the amount of an analyte in a sample comprising contacting a solution comprising said analyte with an enzyme electrode according to any of claims 1-7 and with a standard electrode, applying a potential between said electrodes, and monitoring the electrical current.

14. A method according to claim 13 wherein said analyte is a substrate or an inhibitor for said enzyme.

15. A method according to claim 13 or 14 wherein the analyte gives rise to hydrogen peroxide which is oxidised by the modified electrode to give rise to a signal current, and wherein the analyte contains one or more potentially interfering substances selected from ascorbate, uric acid and paracetamol.

16. A method of determining an analyte in an analyte solution in the presence of one or more potentially interfering substances selected from ascorbate, uric acid and 4-acetamidophenol, comprising contacting said analyte solution with a modified

electrode comprising:

(a) a conductive element having a surface and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface; and with a standard electrode, applying a potential between said electrodes, and monitoring the electrical current.

17. A method of determining an analyte by means of an affinity reaction using an enzyme label, wherein the amount of enzyme label is detected amperometrically by determining a substrate or product thereof by means of a modified electrode comprising:

(a) a conductive element having a surface and

(b) a coating of a hexacyanoferrate-derived material or Prussian blue provided on said surface.

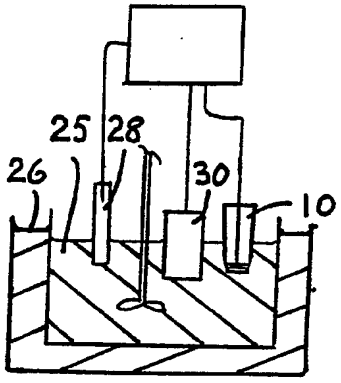

Fig2

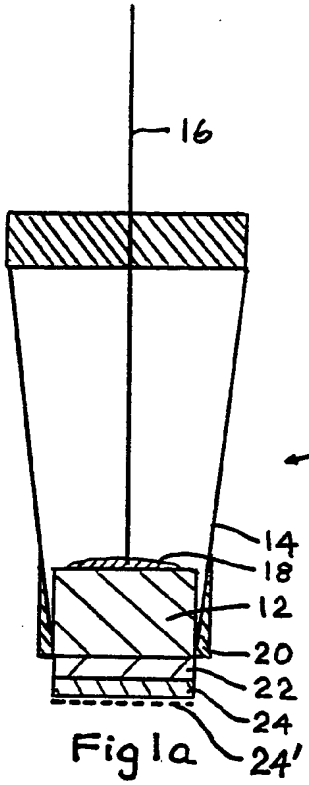

Fig 1a

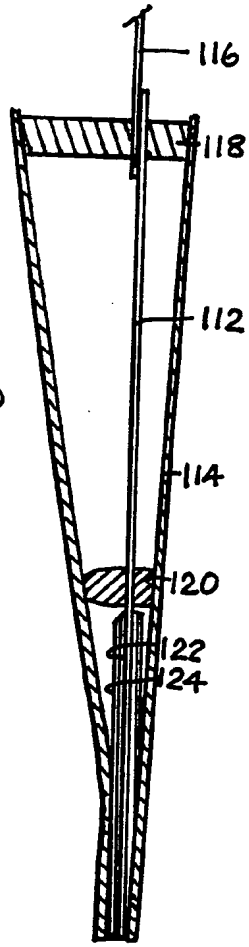

Fig 1b

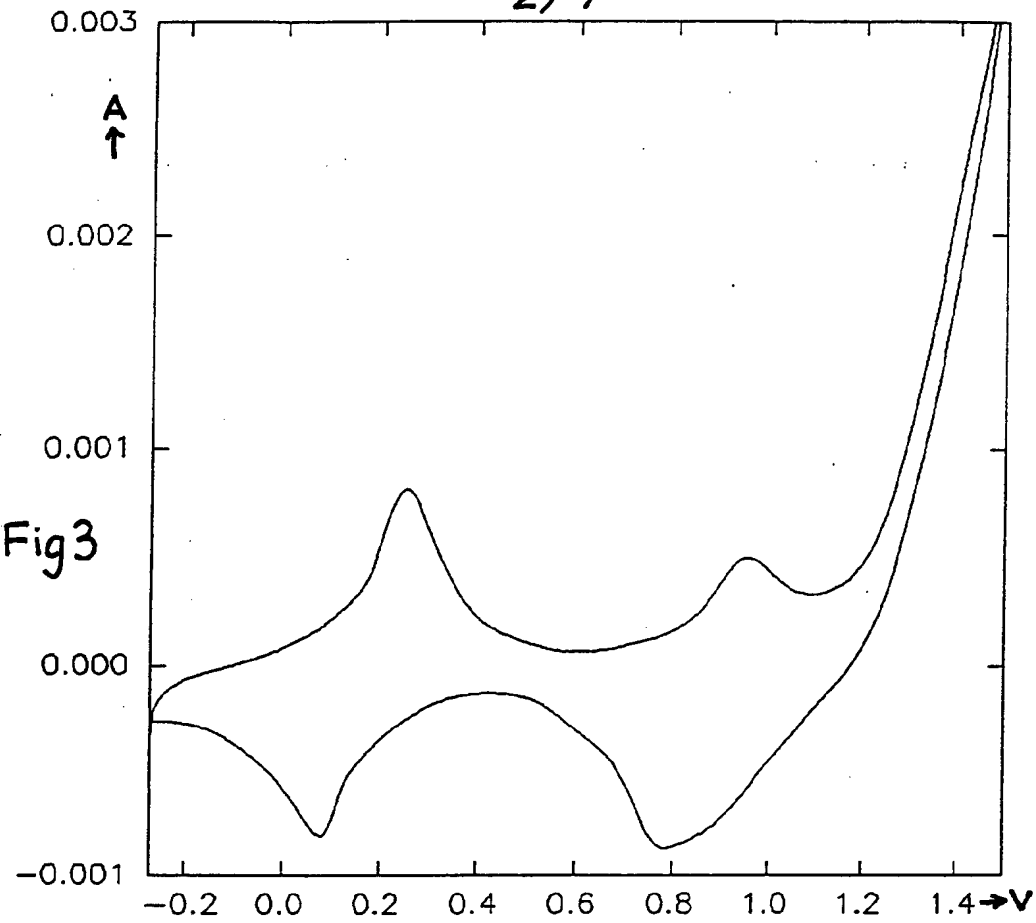
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Fig3

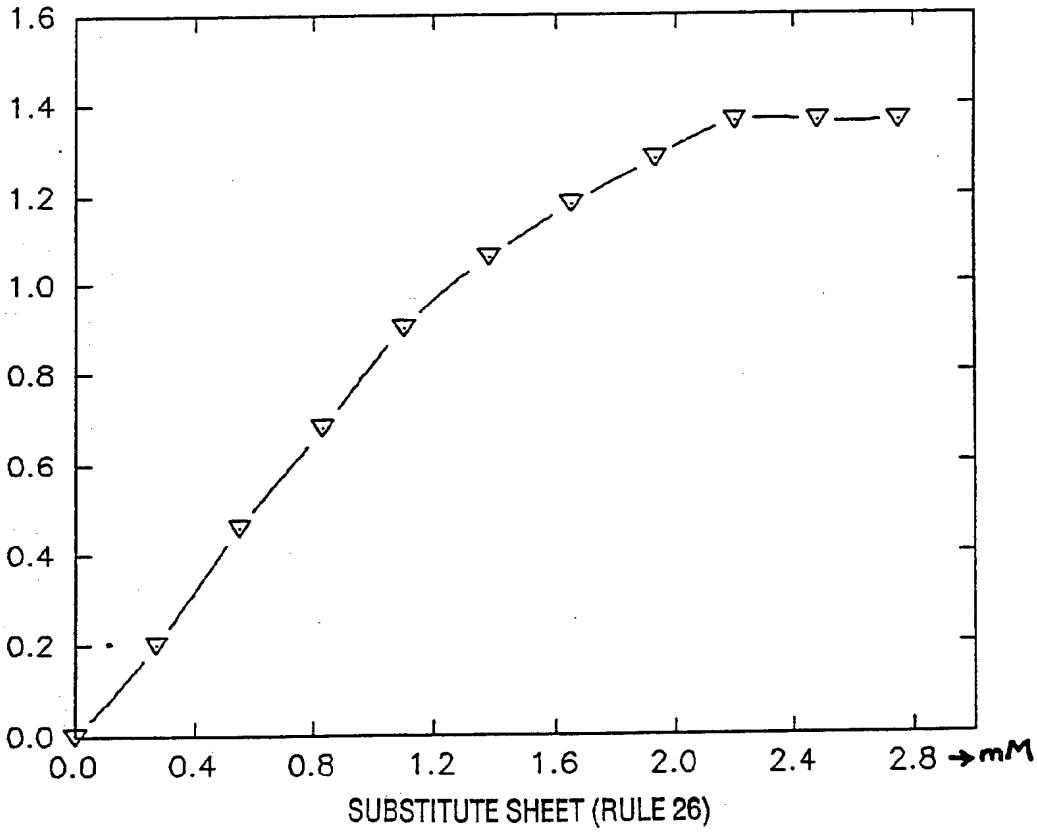
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Fig4

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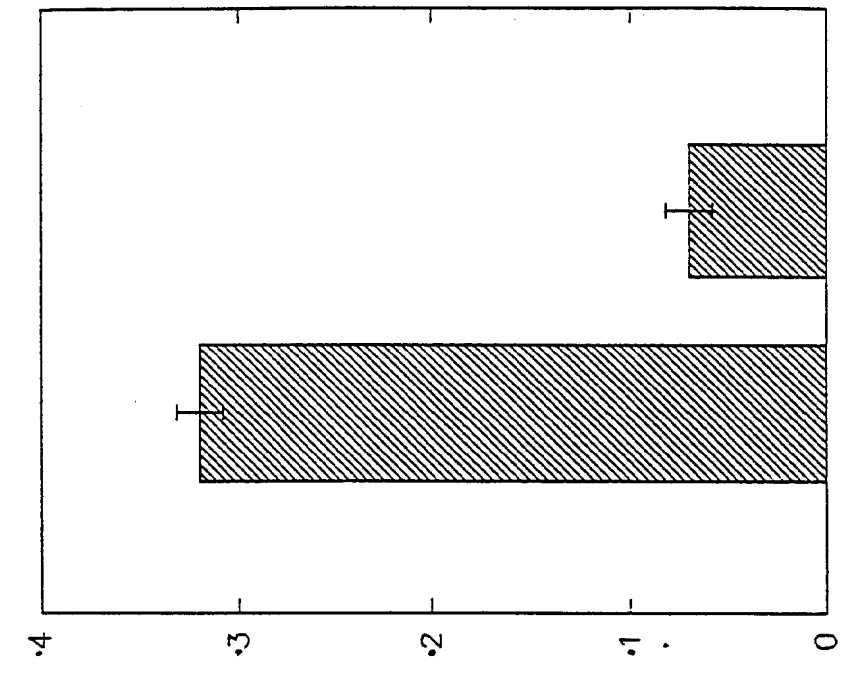

Fig 6

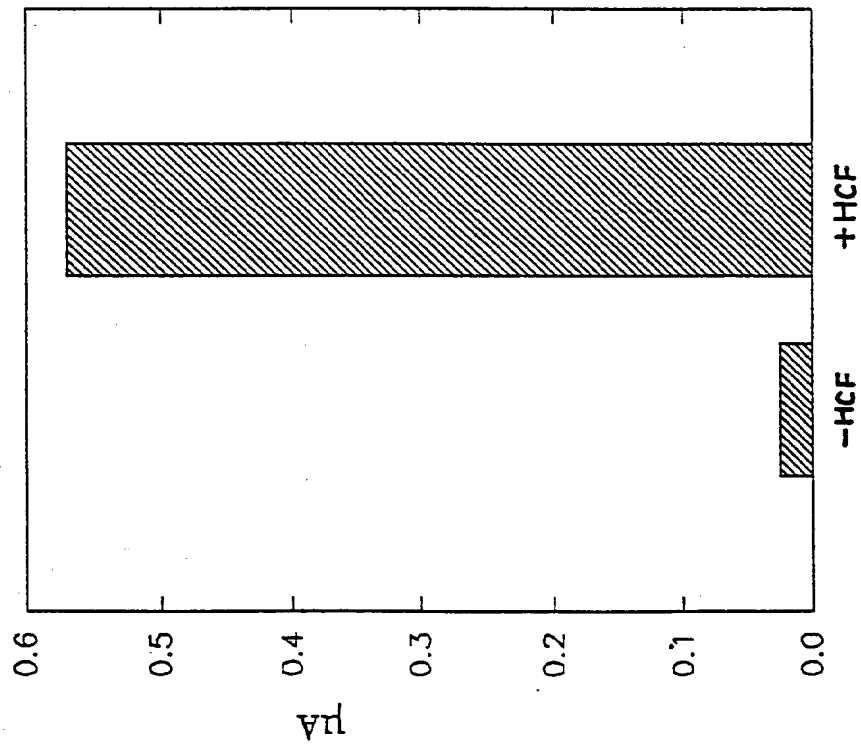

Fig 5

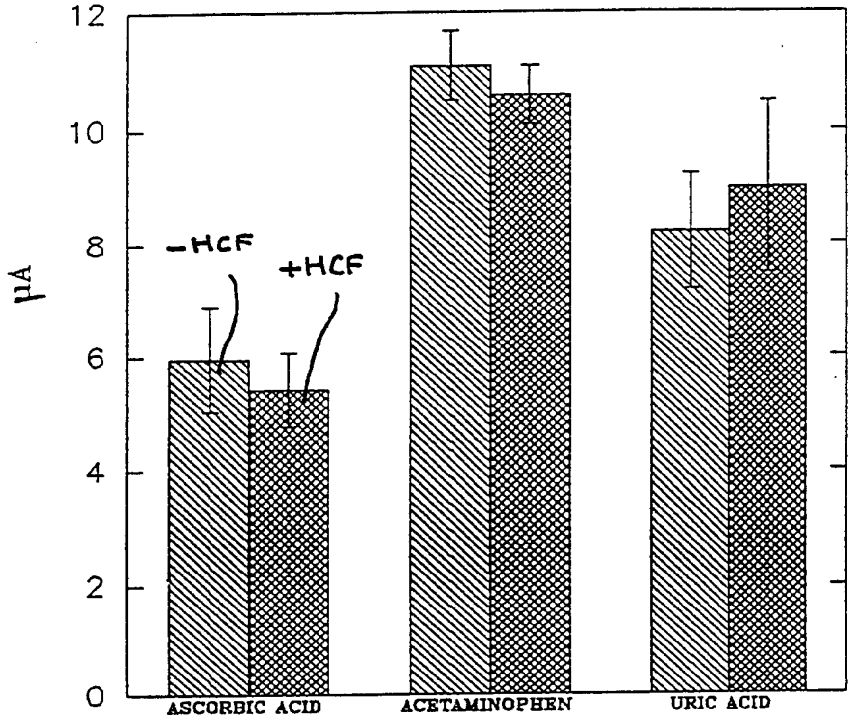
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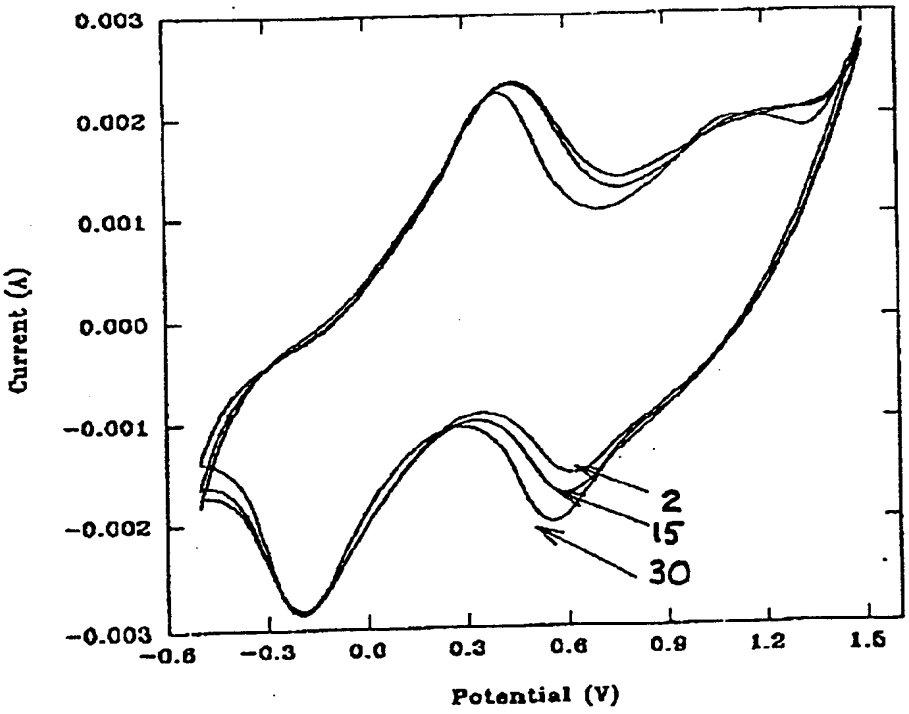
Fig 7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0.001
0.000
-0.001
-0.002
-0.003
Current (A)
-0.6
-0.3
0.0
0.3
0.6
0.9
1.2
1.5
Potential (V)
2
15
30

Fig 11

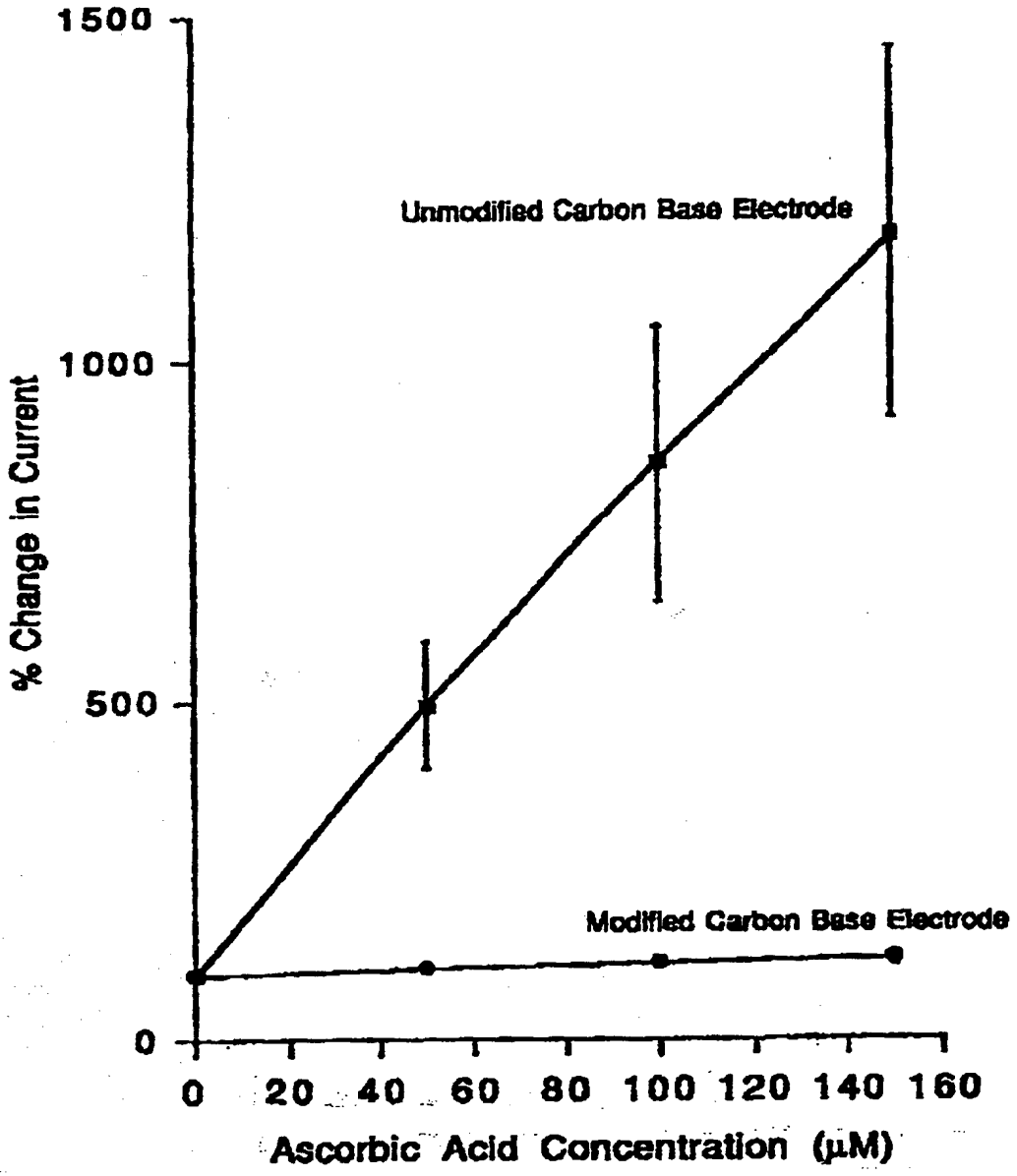
1500
1000
500
0
% Change in Current
Unmodified Carbon Base Electrode
Modified Carbon Base Electrode
0 20 40 60 80 100 120 140 160
Ascorbic Acid Concentration (µM)

Fig 8

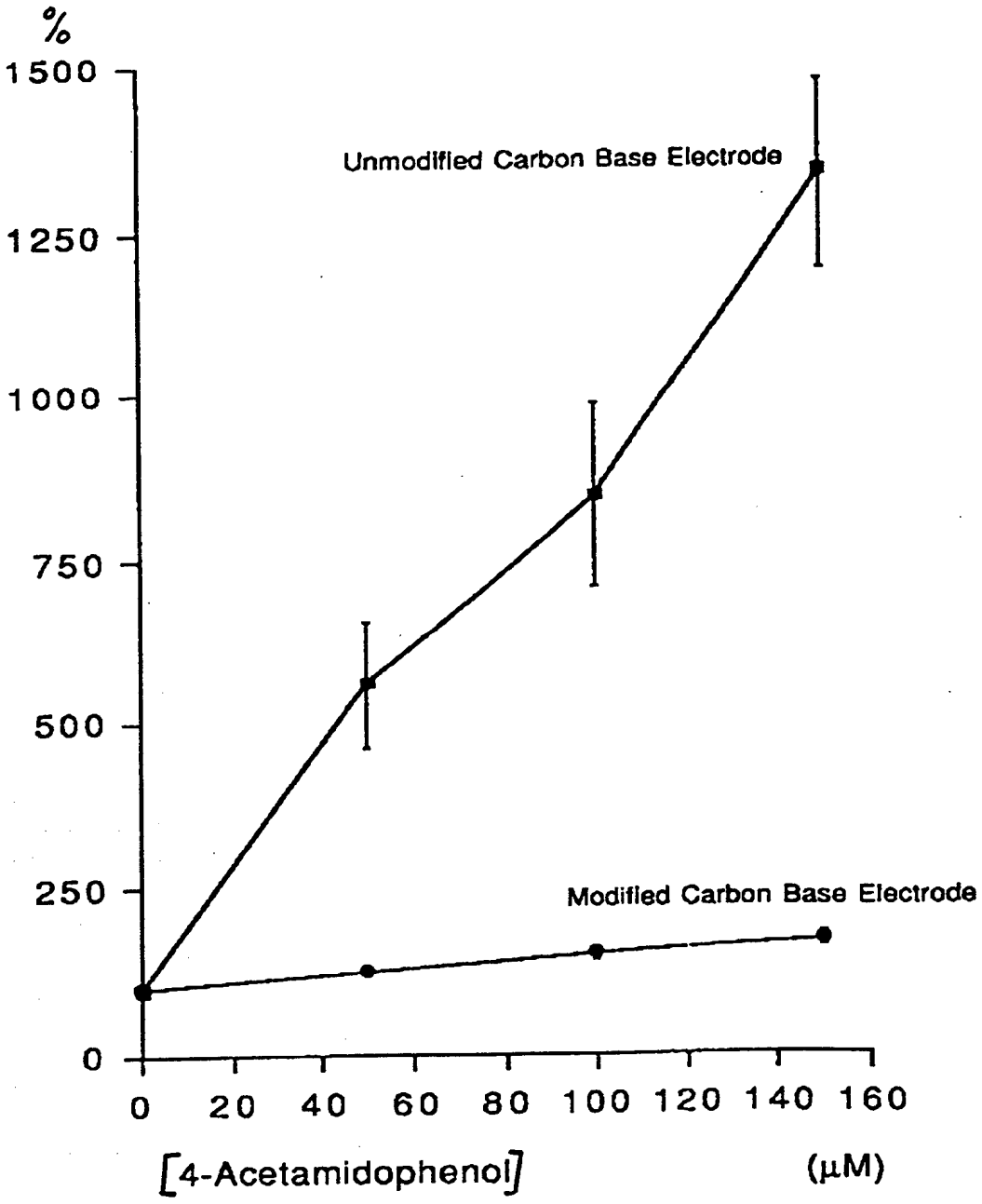
%
1500
1250
1000
750
500
250
0
Unmodified Carbon Base Electrode
Modified Carbon Base Electrode
0 20 40 60 80 100 120 140 160
[4-Acetamidophenol]
(μM)

Fig 9

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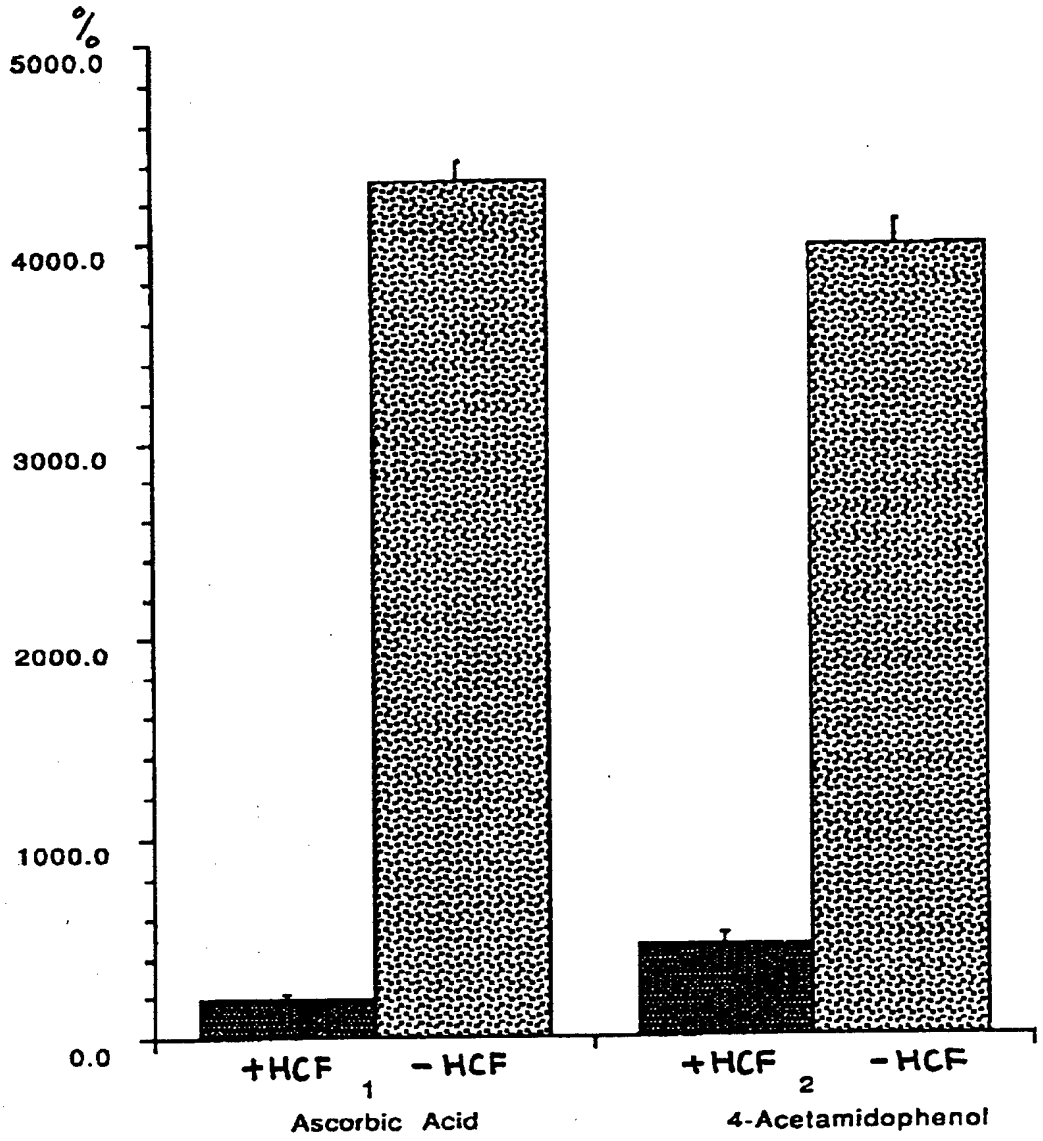
%
5000.0
4000.0
3000.0
2000.0
1000.0
0.0
+HCF
-HCF
1
Ascorbic Acid
+HCF
-HCF
2
4-Acetamidophenol

Fig 10

INTERNATIONAL SEARCH REPORT

International application No. PCT/GB 95/00265

A. CLASSIFICATION OF SUBJECT MATTER
C 12 Q 1/00,G 01 N 27/327,C 12 Q 1/54

According to International Patent Classification (IPC) or to both national classification and IPC 6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C 12 Q,G 01 N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	CHEMICAL ABSTRACTS, vol. 122, no. 3, issued 1995, January 16, (Columbus, Ohio, USA), A.A. KARYAKIN et al."A highly sensitive glucose ampero-metric biosensor based on Prussian Blue modified electrodes", page 450, no. 26 955c; & Anal. Lett. 1994, 27(15), 2861-9. --	1-3, 8-16
A	CHEMICAL ABSTRACTS, vol. 119, no. 7, issued 1993, August 16, (Columbus, Ohio, USA), M. MONTAGNE et al. "Simultaneous use of dehydro-	1,2, 13

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search
02 May 1995

Date of mailing of the international search report
09. 06. 95

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SCHNASS e.h.

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INTERNATIONAL SEARCH REPORT

Internal Application No PCT/GB 95/00265

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	genases and hexacyanoferrate (III)ion in electrochemical biosensors for L-lactate, D-lactate and L-glutamate ions", page 517, no. 67 041h; & Anal. Chim. Acta 1993, 278(1), 25-33.	
A	CHEMICAL ABSTRACTS, vol. 106, no. 9, issued 1987, March 02, (Columbus, Ohio, USA), G.J. MOODY et al. "Chemically immobilized bienzyme electrodes in the redox mediated mode for the flow injection analysis of glucose and hypoxanthine", page 294, no. 63 744y; & Analyst (London) 1987, 112(1), 65-70.	1-3, 13
A	CHEMICAL ABSTRACTS, vol. 92, no. 21, issued 1980, May 26, (Columbus, Ohio, USA), J. MAHENC et al. "Enzyme--specific electrode of glucose based on the amperometric detection of hexacyanoferrate(II)", page 268, no. 176 755p; & C.R. Hebd. Seances Acad. Sci., Ser. C 1979, 289(14), 357-9.	1-3, 13